Open-field ESLIM_007_001

Purpose

The Open Field test is used to assess anxiety and exploratory drive. It is based on the natural tendency of an animal to explore and avoidance reaction to protect itself, which make normal animals to spend more time in the corners and the periphery than in the centre (the most anxiogenic area).

EMPReSS Standard Operating Procedure	Title: Open Field	
	Doc. Number: ESLIM_007_001 Rev No. 1	Date Issued: 22/02/2007

1.0 Purpose:

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2.0 Scope:

2.1 Individuals who have been trained, and are competent in performing the procedures described herein must follow this procedure.

2.2 Any queries, comments or suggestions, either relating to this SOP in general or to a specific problem encountered during a procedure, should be addressed to the project leader.

2.3 Any deviances from this protocol must be reported to the project leader.

3.0 Safety Requirements:

3.1 General laboratory procedures should be followed, which include: no eating, no chewing gum, no drinking, and no applying of cosmetics in the work area. Laboratory coats and gloves must be worn at all times in the work area, unless the protocol specifically describes the appropriate attire for the procedure.

4.0 Associated Documents:

5.0 Notes

5.1 The validity of results obtained from behavioural phenotyping is largely dependent on methods of animal husbandry. It is of vital importance that individuals following this procedure are experienced and aware of the animal's welfare, and is familiar with the animal being tested, in order to reduce the

5.2 The majority of mouse behavioural studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.

5.3 Environmental factors may contribute to the levels of anxiety within the mouse. The temperature, humidity, ventilation, noise intensity and lighting intensity must be maintained at levels appropriate for mice. It is essential that the mice are kept in a uniform environment before and after testing to avoid anomalous results being obtained. In particular, background noise and illumination levels should be measured and documented as environmental conditions in each room. Ideally, all mice should be exposed to the same illumination levels in the holding room (with conventional housing mice in the top of the racks often have up to 10x more Lux than mice in the bottom of racks. In the antechamber and in the testing room no additional experiments which are either noisy or emit odours should be performed during acclimation and testing.

5.4 It is recommended that all phenotyping experimentation is conducted at approximately the same time of day because physiological and biochemical parameters change throughout the day. The ideal testing time for all animals would be in the first half of the lights-on period (usually in the morning until early afternoon).

5.5 Light is an important anxiogenic factor that will strongly influence the ambulation in a U-shaped way (Gray, Fentrop). Experiments under various illumination intensities and factor-analysis have shown that locomotion under dim light is rather a measure of activity than of "fear" (e.g. Trullas & Skolnik 1993). Most studies with pharmacological treatments have shown that anxiolytic agents rather have an effect on the location of the activity than on the amount of activity itself (Crawley & Paylor 1997; Choleris et al. 2001)[1].

6.0 Quality Control:

7.0 Equipment:

7.1 The apparatus is a test arena with walls that can be constructed in different variants. Nevertheless, the following recommendations should be followed, if

possible:

[1] Results are still discussed controversially.

7.1.1 The open field should be square and the arena should not be smaller than 44 x 44cm.

7.1.2 Arena (floor) should not be so smooth that it would prevent the animals from moving freely.

7.1.3 The background (base) colour of the open field should not be white.

7.1.4 The openfield arena(s) is (are) equipped with infrared captors allowing location of the mouse position and evaluation of locomotor activity and rears. Otherwise a videotracking system can be used.

7.3 Illumination system, Luxmeter.

8.0 Supplies:

8.1 Tap water, 50% Ethanol

8.2 Tissue paper

9.0 Procedure:

9.1 **General design**

Mice are tested in automated open fields, each virtually divided into central and peripheral regions. The open fields are placed in a room allowing (if possible) indirect and homogenous illumination (150-200 Lux in the centre of the openfield arena). Each mouse is placed in the periphery of the open field and allowed to explore freely the apparatus for 20 minutes, with the experimenter out of the animal's sight. The distance travelled, the number of rears (if possible), and time spent in the central and peripheral regions are recorded over the test session. The number of entries into the centre, resting time and average speed in each zone of the arena are also recorded.

9.2 **Transportation, acclimation and setting**

9.2.0 Animals are transported in their rack (whenever possible) and left undisturbed for 30 minutes before the test, ideally in an antechamber (pre-test room)

9.2.1 Switch on the computer and set up the appropriate database in the programme so that mouse activity is monitored for a 20 minute period. Ensure that lighting conditions are as desired (150-200 Lux in the centre of the arena) and that all equipment is working correctly.

9.3 Testing

9.3.1 Testing is conducted during the light phase of the cycle with 1 hour gap from the light/dark change.

9.3.2 Wipe the apparatus clean with 50% alcohol and allow time for it to dry.

9.3.3 Each mouse is placed in the middle of a peripheral zone of the arena facing the wall and allowed to explore freely the apparatus for 20 min, with the experimenter out of the animal's sight. If more than one mouse can be tested in parallel, in adjacent open field arenas and mice are video-tracked, it is important to ensure that the tracking of each mouse starts as soon as the mouse is released to make data comparable.

9.3.4 At the end of the run, animals are labelled (if necessary) and put back into their home cage.

9.3.5 After each run, any faeces are removed and the arena is thoroughly wiped (water then alcohol 50 %).

10.0 Data Records and Reports:

10.1 Zones: arena is subdivided into central and peripheral "border" zones (The defined border of the arena is 8cm wide, the central area about 40 (35-45 % of total).

10.2 Parameters recorded:

10.2.1 Total distance travelled and its evolution per 5-min periods in the whole arena.

10.2.2 Total distance in the central and peripheral zones

10.2.3 If possible, total number of rears and its evolution per 5-min periods in the whole arena

10.2.4 Percentage of time spent in the central zone and its evolution per 5-min periods.

10.2.5 Percentage of distance in the central zone and its evolution per 5-min periods.

10.2.6 Duration of the immobility (resting time in seconds) in the whole arena, in the periphery and in the center.

10.2.7 Average speed in the whole arena, in the periphery and in the center.

10.2.8 Number of entries in the central zone.

10.2.9 Latency to the first entry into the central zone, if possible.

11.0 Supporting information:

11.1 Crawley JN, Paylor R (1997) A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. Horm Behav 31:197-211

11.2 Choleris E, Thomas AW, Kavaliers M, Prato FS (2001) A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci Biobehav Rev 25:235-260

11.3 Fentron N (2003) Auswirkungen eines Defizits des Neuronalen

Zelladhäsionsmoleküls (*NCAM*) im Telencephalon auf Lernen, Gedächtnis und

Individualität bei einer gentechnisch veränderten Labormaus, 1. Auflage, *Litis Press Publishers*, München, download: http://www.fentrop.com/nikolas/publications, [13.11.2003]

11.4 Fentrop N, Wotjak CT (2000) Fiat lux! Spotting a common experimental problem, Poster presented at *Measuring Behavior 2000*, 3rd International Conference on Methods and Techniques in Behavioral Research, 15-18 August 2000, Nijmegen, The Netherlands

11.5 Gray JA (1987) The Psychology of Fear and Stress. 2 edn. Cambridge: - Cambridge University Press.

11.6 Trullas R, Skolnick P (1993) Differences in fear motivated behaviors among inbred mouse strains. Psychopharmacology 111:323-331

12.0 History review:

12.1 Testing duration is reduced to 20 min (instead of 30 min in the SOP document 10_001, Revision 0, Date issued 01/06/04)

13.0 Emergency Procedures:

Parameters and Metadata

Distance travelled ESLIM_007_001_001 | v1.0

seriesParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: cm

Description: Distance_travelled

Increments: 5, 10, 15, 20,

Number of rears ESLIM_007_001_002 | v1.0

seriesParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Description: Number_of_rears

Increments: 5, 10, 15, 20,

Whole arena resting time ESLIM_007_001_003 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: s

Description: Whole_arena_resting_time

Whole arena permanence time ESLIM_007_001_004 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: s

Description: Whole_arena_permanence_time

Whole arena average speed ESLIM_007_001_005 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: cm/s

Description: Whole_arena_average_speed

Periphery distance travelled ESLIM_007_001_006 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: cm

Description: Periphery_distance_travelled

Periphery resting time ESLIM_007_001_007 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: s

Description: Periphery_resting_time

Periphery permanence time ESLIM_007_001_008 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: s

Description: Periphery_permanence_time

Periphery average speed ESLIM_007_001_009 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: cm/s

Description: Periphery_average_speed

Centre distance travelled ESLIM_007_001_010 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: cm

Description: Centre_distance_travelled

Centre resting time ESLIM_007_001_011 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: s

Description: Centre_resting_time

Centre permanence time ESLIM_007_001_012 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: s

Description: Centre_permanence_time

Centre average speed ESLIM_007_001_013 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: cm/s

Description: Centre_average_speed

Latency to centre entry ESLIM_007_001_014 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** true **Is Annotated:** true

Unit Measured: s

Description: Latency_to_centre_entry

Number of centre entries ESLIM_007_001_015 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Description: Number_of_centre_entries

Distance travelled - total ESLIM_007_001_701 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: cm

Description: Distance_travelled__total

Derivation: archived('sum_of_increments(ESLIM_007_001_001)')

Number of rears - total ESLIM_007_001_702 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: number

Description: Number_of_rears__total

Derivation: archived('sum_of_increments(ESLIM_007_001_002)')

Percentage centre time ESLIM_007_001_703 | v1.0

simpleParameter

Req. Analysis: false **Req. Upload:** false **Is Annotated:** true

Unit Measured: %

Description: Percentage_centre_time

Derivation: archived('(ESLIM_007_001_012/ESLIM_007_001_004)x100')

Equipment name ESLIM_007_001_801 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Description: Equipment_name

Equipment manufacturer ESLIM_007_001_802 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Description: Equipment_manufacturer

Equipment model ESLIM_007_001_803 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Description: Equipment_model

Central zone surface area ESLIM_007_001_804 | v1.0

procedureMetadata

Req. Analysis: true **Req. Upload:** true **Is Annotated:** false

Unit Measured: % of total area

Description: Central_zone_surface_area

Light intensity in the centre of the arena ESLIM_007_001_805 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** true **Is Annotated:** false

Unit Measured: Lux

Description: Light_intensity_in_the_centre_of_the_arena

Number of animals per cage ESLIM_007_001_806 | v1.0

procedureMetadata

Req. Analysis: false **Req. Upload:** false **Is Annotated:** false

Unit Measured: number

Description: Number_of_animals_per_cage